REMARKS

Favorable reconsideration is respectfully requested.

The claims are 1, 2, 5, 7 and 9 to 12.

Claims 1, 2, 5, 7 and 9 to 12 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yanagawa (U.S. 6,197,328) or Yanagawa (EPO 0 681 833 A2).

This rejection is respectfully traversed.

1. Yanagawa (U.S. 6,197,328 B1), hereinafter referred to as US '328 is based on USSN 09/377,905 which was filed on August 20, 1999, and Yanagawa (EPO 0 681 833 A2), hereinafter referred to as EP '833 is an EP application which claims priority based on JP 120778/94 filed on May 11, 1994. Both of these references name Dr. Yanagawa as a single inventor.

It is disclosed in US '328, column 1, lines 30–40, as follows:

> As a result of intensive studies, he has proposed nasally administrable composition using a unique carrier, wherein the physiologically active peptide is dispersed and absorbed homogenously onto the carrier, and is highly absorbable into the body via nasal route.
> Through further extensive studies and researches, the inventor of the present invention has found that the amounts of absorbed physiologically active compound into a body via nasal route well proportion to size of surface area of the carrier. (emphasis added)

It is presumed from the above-quoted passage that Dr. Yanagawa completed his invention of US '328 about five years after he completed his invention of EP '833.

In detail, the following is assumed: as the rejection points out, Dr. Yanagawa found that the use of calcium carbonate with a particle surface area from 0.1 m^2/g to 0.4 m^2/g as a carrier achieved excellent absorption of medicine such as insulin (see US '328, column 3, lines 48–55), and further that a "unique mixed carrier of calcium carbonate and HPC-H" was a "preferable mode of invention", and thus claimed such mode in US '328.

As is seen from the above-quoted passage of US '328, Dr. Yanagawa had found that the amounts of absorbed physiologically active compound into a body via nasal route well proportion to size of surface area of the carrier.

On the other hand, the Final Rejection, page 4, line 3 from the bottom, to page 5, line 5 states:

> The surface area taught is slightly less than applicant's claimed surface area, however, it would have been obvious to one of ordinary skill in this art that suitable ranges or amounts could be determined through routine or manipulative experimentation to obtain the best possible results, as these are indeed variable parameters. (emphasis added)

Hence, if the rejection is right in the above comments, it would be reasonable to consider that Dr. Yanagawa not only invented calcium carbonate fine particulate with a particle surface area from 0.1 m^2/g to 0.4 m^2/g, but must have gone further and studied applicant's claimed calcium carbonate fine particulate with a slightly larger particle surface area and must have thus attained the present invention.

Actually, however, Dr. Yanagawa did not direct his efforts for improvement towards calcium carbonate fine particulates *per se* which would have better actions, as did present Applicants. Dr. Yanagawa instead provided a formulation for the nasal administration of medicines such as insulin, which comprises a combination of calcium carbonate particulate and a cellulose derivative containing, as absorption accelerator, HPC-H (high substituted hydroxypropylcellulose). (see US '328).

Attention should be paid to the fact that even Dr. Yanagawa who, for the first time, used calcium carbonate particulates for carrier combined, at a later time, calcium carbonate particulate with absorption accelerator for the sake of improvement of absorptivity of medicines into an organism while employing a particle surface area of calcium carbonate particulate from 0.1 m^2/g to 0.4 m^2/g and not trying to change this particle surface area even after five years' research for the improvement from the time when he employed calcium carbonate particulate.

Therefore no art-skilled person who has read EP '833 and US '328 would have foreseen that calcium carbonate fine particulates with a particle surface area larger than "from 0.1 m^2/g to 0.4 m^2/g" would be readily available, and that the single use of such a carbonate fine particulate would give a formulation, with excellent action and effects, for the nasal absorption of medicines such as insulin, without using an absorption accelerator in combination.

2. Reference is now made to Dr. Shunji Haruta's Declaration which was cited in the response dated May 11, 2004, to the Office Action dated November 12, 2003. Figure 2 attached to said Declaration gives values of C_{max} (μU/mL) of mean serum insulin concentration which are obtained when Yanagawa's formulation prepared according to US '328 and applicant's formulation are each intranasally administered to healthy human volunteers. Said values are as follows:

Formulation	C_{max} (μU/mL)	
Yanagawa's Formulation		
100 IU/50 mg $CaCO_3$	about 5.5 [= (8 + 3)/2]	N = 2
50 IU/50 mg $CaCO_3$/5 mg HPC-H	about 19 [= (20 + 18)/2]	N = 2
Applicant's Formulation		
48 IU/48 mg PS-$CaCO_3$	about 31.3	N = 6

In Yanagawa, as is seen from the above, a formulation of 50 IU/50 mg $CaCO_3$/5 mg HPC-H whose carrier is a combination of $CaCO_3$ and HPC-H as an absorption accelerator shows a C_{max} (μU/mL), i.e., maximum serum concentration of medicine, of about 3.5 times (= 19/5.5) as high as C_{max} (μU/mL) of a formulation of 100 IU/50 mg $CaCO_3$ whose carrier consists of $CaCO_3$ alone, although the amount of medicine loaded on a carrier in the former formulation is smaller than in the latter. This means that the use of HPC-H as an absorption accelerator in combination with $CaCO_3$ achieves a high C_{max} (μU/mL).

In the present invention, on the other hand, a formulation of 48 IU/48 mg PS-$CaCO_3$ whose carrier consists of $CaCO_3$ alone shows a C_{max} (μU/mL) of about 5.7 times (= 31.3/5.5), or, in consideration of the difference in the amount of insulin administered, even about 11.9 times (= (31.3/5.5) × (100 IU/48 IU)), as high as C_{max} (μU/mL) of Yanagawa's formulation of 100 IU/50 mg $CaCO_3$ whose carrier consists of $CaCO_3$ alone. This improvement attained by the present invention would have been quite unforeseeable from the action and effect of the particle surface area of "from 0.1 m^2/g to 0.4 m^2/g" which Dr. Yanagawa found from his five-year study after US '328 was filed. Furthermore, Applicant's formulation shows a C_{max} (μU/mL) of about 1.6 times (=

31.3/19) as high as C_{max} (μU/mL) of even Yanagawa's formulation of 50 IU/50 mg $CaCO_3$/5 mg HPC-H which is disclosed in Yanagawa as a preferable embodiment.

No one of ordinary skill in the art would have thought that a formulation of such a high C_{max} (μU/mL) of medicine would be provided by the present invention whose calcium carbonate fine particulate was, according to the rejection, not significantly different, in surface area, from the carrier of US '328.

3. Further comparison between calcium carbonate fine particulate used in the present invention and calcium carbonate fine particulate used in Yanagawa is as follows:

The paper (DIABETES TECHNOLOGY & THERAPEUTICS) attached hereto is a report of Applicant on the utility of Applicant's formulation in the nasal administration of insulin. Fig. 1 of this paper shows electron micrographs of calcium carbonate which is used in Applicant's formulation and of regular calcium carbonate which is used in Yanagawa's formulation (which was obtained from Sumida Shokai (Tokyo, Japan) owing to information provided by Dr. Yanagawa; also see the last line of Table-1 of Dr. Haruta's Declaration). As is clearly seen in said electron micrographs, calcium carbonate which is used in Applicant's formulation has a special particulate structure as defined in Claim 2 of the present application, which is different from the structure of regular calcium carbonate.

As stated in the above 2., Applicant's formulation shows a C_{max} (μU/mL) of about11.9 times as high as C_{max} (μU/mL) of Yanagawa's formulation. This dramatic improvement in serum concentration of insulin is due to the fact that calcium carbonate which is used in Applicant's formulation is very different in particulate structure from regular calcium carbonate.

Such being the case, the present invention is unobvious over US '328 and EP '833.

No further issues remaining, allowance of this application is respectfully requested.

If the Examiner has any comments or proposals for expediting prosecution, please contact undersigned at the telephone number below.

Respectfully submitted,

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DIABETES TECHNOLOGY & THERAPEUTICS
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An Effective Absorption Behavior of Insulin for Diabetic Treatment Following Intranasal Delivery Using Porous Spherical Calcium Carbonate in Monkeys and Healthy Human Volunteers

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ABSTRACT

Porous spherical calcium carbonate (PS-$CaCO_3$), in contrast to regular calcium carbonate ($CaCO_3$), which has a cuboidal particle shape, has a characteristic spherical particle shape with a large number of porous, sliver crystals. The effect of PS-$CaCO_3$ as a drug carrier on intranasal insulin absorption was investigated in cynomolgus monkeys and healthy human volunteers. Each insulin formulation (powder) containing PS-$CaCO_3$ or regular $CaCO_3$ was administered intranasally. Serum insulin and glucose levels after administration were evaluated. The insulin absorption after intranasal administration with each $CaCO_3$ was found to be much more rapid than that after subcutaneous administration. The serum insulin level after intranasal insulin delivery (16 U per monkey) with PS-$CaCO_3$ showed a higher C_{max} (403.5 μU/mL) and shorter T_{max} (0.167 h) when compared with regular $CaCO_3$. The serum glucose level reduction rate after intranasal delivery using PS-$CaCO_3$ was faster than that of regular $CaCO_3$, reflecting the difference in absorption rates. Following repeated intranasal administrations for 4 weeks in monkeys, no toxicity was observed even with a maximum insulin dose level of 25 U. Furthermore, the intranasal insulin absorption rate with PS-$CaCO_3$ in healthy humans was also observed to be considerably faster than that with regular $CaCO_3$. Effects of PS-$CaCO_3$ on a more effective absorption behavior of insulin were considered to be the result of a greater affinity between the nasal mucosa layer and PS-$CaCO_3$, which is closely related to its structural characteristics. Thus, intranasal insulin delivery using PS-$CaCO_3$ is thought to be a safe and highly available system enabling more effective insulin absorption behavior with the appearance of endogenous postprandial insulin secretion in healthy humans. We believe that our intranasal insulin delivery system enabling a rapid and short-acting pharmacological effect against postprandial hyperglycemia will be more beneficial than pulmonary insulin delivery systems in the treatment of diabetes.

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INTRODUCTION

DIABETIC PATIENTS usually require painful single or multiple daily injections of insulin, leading to low compliance of patients' medical regimens. To develop a promising insulin delivery system as an alternative to subcutaneous injection, a number of investigations into alternative routes of insulin delivery, including inhalation,[1] intranasal,[2] transdermal,[3] oral,[4] buccal,[5] and rectal[6] administration, have been performed.

Currently, major pharmaceutical companies are vigorously developing pulmonary insulin delivery systems and have advanced these to late stages in clinical trials.[7–9] However, the chronic pulmonary insulin delivery may include a concern about pulmonary fibrosis. The use of injectable insulin formulations (fast-acting products) for postprandial hyperglycemia often leads to a hypoglycemic side effect due to excessive duration of the blood insulin concentration.

It is reported that insulin administered intranasally shows rapid and short-term absorption, resulting in a rapid and short-acting pharmacological effect, and is likely to reproduce endogenous postprandial insulin secretion in healthy humans.[10] Intranasal insulin delivery should, therefore, lead to both a shortening of the onset of post-dosing pharmacological effects and the reduction of hypoglycemic side effects. Although most attempts at intranasal insulin delivery have achieved adequate absorbability by utilizing absorption enhancers,[11,12] these have not yet led to clinical use owing to possible toxicological effects. Yanagawa[13] suggested that regular calcium carbonate ($CaCO_3$) is a safe and viable drug carrier for the intranasal delivery of various drugs including insulin. It is shown in a separate report that compared with liquid formulation, regular $CaCO_3$ powder as a carrier improves the intranasal bioavailability of elcatonin.[14]

In the present study, porous spherical (PS-) $CaCO_3$ having a characteristic particle shape, as an intranasal drug carrier for the treatment of postprandial hyperglycemia in diabetes, was evaluated in cynomolgus monkeys and human volunteers, and compared with regular $CaCO_3$.

MATERIALS AND METHODS

Materials

Human recombinant insulin (fast-acting, water-insoluble product) for human intranasal administration (potency 26.7 U/mg) and insulin for animal nasal administration (potency 28.7 U/mg) were purchased from Novo-Nordisk A/S (Bagsvaerd, Denmark) and Intergen Ltd. (New York, NY), respectively. Human recombinant insulin solution (fast-acting, water-soluble product) used for subcutaneous study [Novolin® R injection (potency 40 U/mL)] was purchased from Novo-Nordisk A/S. Regular $CaCO_3$ was purchased from Sumida Shokai (Tokyo, Japan). PS-$CaCO_3$ was purchased from Tsutsumi Techno Planning (Tokyo). PS-$CaCO_3$ has a characteristic spherical particle shape with a large number of porous, sliver crystals produced by a specialized manufacturing process. Other chemicals and reagents were purchased commercially.

Animals

Male cynomolgus monkeys (Shin Nippon Industries, Kagoshima, Japan), weighing 3.7–4.2 kg, were used. Prior to the experiment the animals were fasted overnight; however, water was available ad libitum. The animals were rested for at least 2 weeks between experiments. The animal study was performed after receiving approval from the Ethics Committee of Shin Nippon Biomedical Laboratories Ltd.

Volunteers

Sixty-five healthy male volunteers 21–42 years old participated in the study after returning informed consent forms. The protocol for the study was approved by the Ethics Committee of CPC Clinic. Each subject was dosed after a 12-h overnight fast.

Characterization of calcium carriers

The shape and surface characteristics of each calcium carrier were investigated by scanning electron microscopy (model JSM-5200, JEOL Ltd., Tokyo). The specific surface area of each calcium carrier was measured by Brunauer-

Emmett-Teller and nitrogen gas adsorption methods.

Preparation of the insulin formulation

Regular $CaCO_3$ and PS-$CaCO_3$ within the particle size range of 20–32 μm were divided by sieving. The resultant material was mixed well for 5 min after the addition of insulin powder and purified water, and the final insulin formulation was prepared after freeze-drying. Mixtures of 32 mg (for monkeys) and 32 or 48 mg (for humans) were loaded into HPMC capsules (Shionogi Qualicaps Ltd., Nara, Japan). Six types of intranasal insulin formulation were prepared: 16 U/32 mg of insulin/regular $CaCO_3$ and 16 U/32 mg of insulin/PS-$CaCO_3$ (for monkeys); 16 U/32 mg of insulin/regular $CaCO_3$ and 16 U/32 mg, 48 U/48 mg, and 64 U/48 mg of insulin/PS-$CaCO_3$ (for humans). Insulin levels in the capsules were confirmed to be between 95% and 105% and to be stable for at least 6 months after preparation, at 25°C and 60% humidity, by a high-performance liquid chromatography determination of human insulin based on USP.

Toxicity study

Before the clinical study, toxicity studies of our intranasal insulin formulation, including urine and blood tests, testicular toxicity tests, mutagenicity tests, and local irritation tests (nasal mucosa), etc., were performed with repeated administrations to cynomolgus monkeys. Insulin was administered intranasally once a day for 4 weeks at doses of 0/0 (air control), 0/25, 16/16, 20/20, and 25/25 (U of insulin/mg of calcium carrier). In local irritation tests of nasal mucosa, nasal epithelia (olfactory and respiratory area) were observed by optical microscopy under hematoxylin and eosin staining. During and/or after repeated administrations over 4 weeks, we observed body weights and performed urine tests (color, pH, glucose, ketone body, bilirubin, urine occult blood, urobilinogen, protein, etc.), hematology tests (erythrocyte count, leukocyte count, hematocrit value, hemoglobin concentration, blood platelet count, mean corpuscular volume, mean corpuscular hemoglobin, etc.), and serum biochemistry tests (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatine phosphokinase, total bilirubin, total protein, etc.).

Device for intranasal administration

Each insulin formulation was administered intranasally with a nasal spray device (Jetlizer®, Unisia Jecs Corp., Gunma, Japan). This hand-operated device disperses the powder as widely as possible onto the nasal mucosa. A capsule containing the appropriate dose is loaded into the device. Thin needles built into the device pierce the top and bottom of the capsule. Pressurized air is then forced through the capsule by pressing an air pump of the device. The insulin formulation is gathered up by the pressurized airflow and released from the capsule. It was confirmed that >98% of the contents of the capsules was released.

Insulin administration and blood samples

Subcutaneous administration. In the animal study, insulin solution was administered subcutaneously in the neck at a dose level of 0.5 U per monkey. Blood (1.5 mL) was drawn from the femoral vein 0, 10, 20, 30, 40, 60, 120, and 240 min after administration.

In the clinical study, insulin solution was administered subcutaneously in the abdomen at a dose level of 4.0 U per human subject. Blood (4.0 mL) was drawn from the antecubital vein 0, 5, 15, 30, 45, 60, 90, 120, 180, 240, and 360 min after administration.

Intranasal administration. In the animal study, each insulin formulation was administered intranasally at a dose level of 16 U per monkey using the exclusive device. Blood (1.5 mL) was drawn from the femoral vein 0, 10, 20, 30, 40, 60, 120, and 240 min after administration.

In the clinical study, each insulin formulation was administered at dose levels of 16, 48, and 64 U per human subject using the device. Blood (4.0 mL) was drawn from the antecubital vein 0, 5, 15, 30, 45, 60, 90, 120, 180, 240, and 360 min after administration.

Assay method

Serum was separated by centrifugation at 4°C and 1,600 *g*, and then stored at −20°C

until analysis. Serum glucose levels were determined by the glucokinase/glucose 6-phosphate dehydrogenase method (Iatron Laboratories, Ltd., Tokyo). Serum insulin levels were determined by the enzyme-linked immunoassay method, using an Abbott IMx analyzer (Abbott Laboratories, North Chicago, IL). The coefficient of variance of the standard curve ranged from 3.09% to 3.21%. The squared correlation coefficient was >0.997.

Pharmacokinetic parameters

The maximum serum concentration (C_{max}) and the time to reach C_{max} (T_{max}) were recorded for each observed serum insulin concentration profile. The area under the serum concentration–time curve (AUC) was calculated by the trapezoidal method.

The relative bioavailability (BA) of intranasal insulin delivery was calculated in comparison with subcutaneous administration and was expressed as $BA = Dose_{sc}/Dose_{nasal} \times AUC_{nasal}/AUC_{sc} \times 100$. The relative BA in monkey studies was calculated using each AUC for 0–4 h after dosing and was expressed as $BA_{0-4\ h}$. The relative BA in human studies was calculated using each AUC for 0–1 h or 0–6 h after dosing to evaluate the relation between the effective absorbing period for the intranasal administration and BA, and was expressed as $BA_{0-1\ h}$ and $BA_{0-6\ h}$, respectively.

Statistical analysis

Statistical significance was evaluated by Student's t test. Results were expressed as the mean ± SD of at least six experiments.

RESULTS

Characterization of calcium carriers

Electron micrographs of regular $CaCO_3$ and PS-$CaCO_3$ are shown in Figure 1. The particle shape and surface structure of regular $CaCO_3$ are of a latticed cuboid with smooth surfaces. In contrast, those of PS-$CaCO_3$ are spherical with a rough, porous surface. As shown in the differences in structural characteristics, the measured specific surface area of PS-$CaCO_3$ (1.99 m^2/g) was approximately 15 times greater than that of regular $CaCO_3$ (0.12 m^2/g). The difference between the structural characteristics of the two calcium carriers was thought to influence their mutual contact properties with the insulin and nasal mucosa.

Toxicity study

In the repeated intranasal administrations for 4 weeks, local toxicity to the nasal epithelium (olfactory and respiratory areas) was not observed even when insulin was administered intranasally at a maximum dose level of 25 U. In urine, hematology, and serum biochemistry

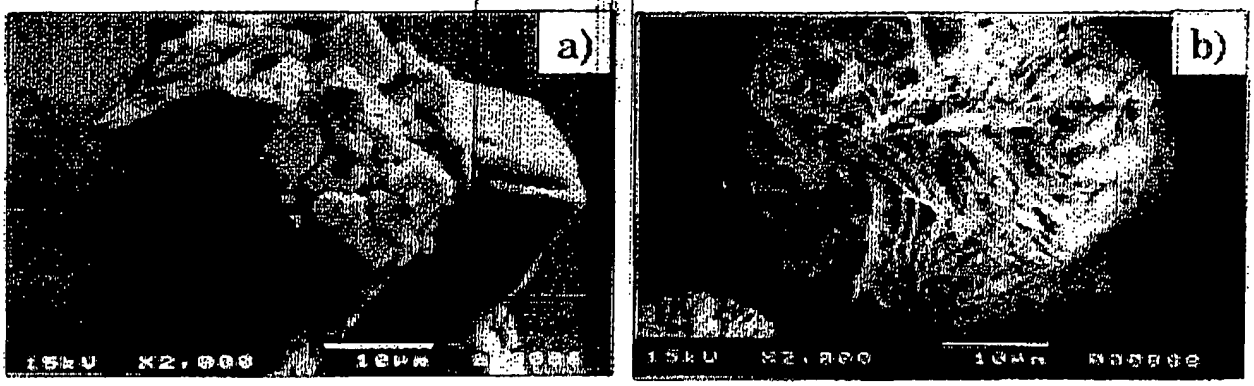

FIG. 1. Electron micrographs of calcium carriers: (a) regular $CaCO_3$ at ×2,000 magnification and (b) PS-$CaCO_3$ at ×2,000 magnification.

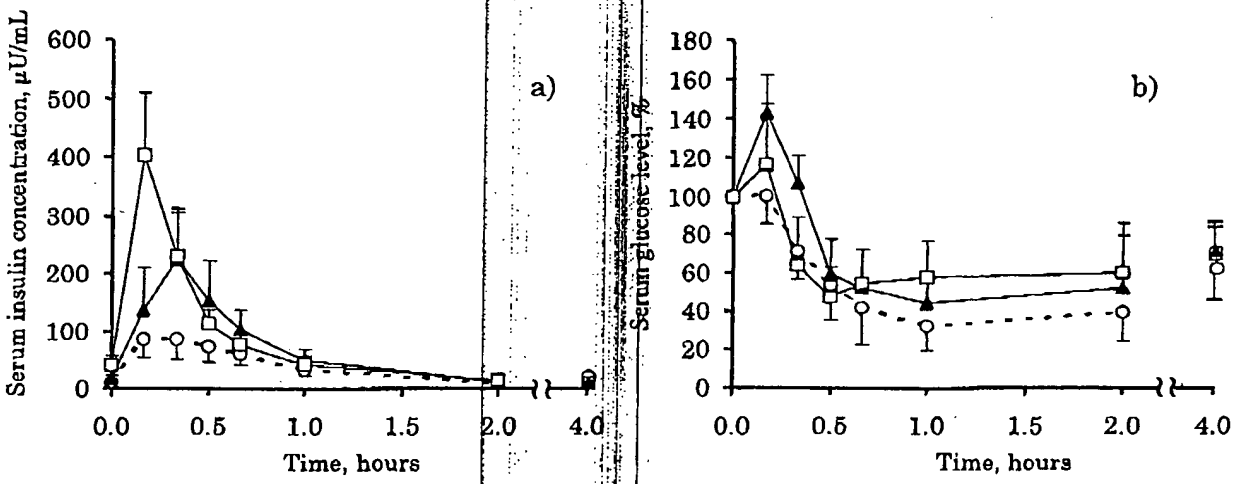

FIG. 2. Serum insulin concentration curves (a) and serum glucose level curves (b) after subcutaneous and intranasal administration in monkeys. Doses of insulin administered subcutaneously and intranasally were 0.5 and 16 U per monkey, respectively. Results are expressed as mean ± SD of six experiments. Serum glucose levels are expressed as the ratio of serum glucose concentration to the initial concentration. Each insulin formulation is expressed as follows: (open circle) subcutaneous formulation; (solid triangle) intranasal formulation with regular $CaCO_3$; (open square) intranasal formulation with PS-$CaCO_3$.

tests, no apparent differences were also observed between the control animals and those treated with the calcium carrier with and without insulin for 4 weeks. In the other toxicity studies, no apparent differences were also observed in comparison with control animals. These suggested safety of our intranasal insulin delivery system with the calcium carrier.

Animal study

Figure 2 shows the average serum concentration profiles of insulin and glucose after subcutaneous or nasal administration with regular $CaCO_3$ and nasal administration with PS-$CaCO_3$. The corresponding pharmacokinetic parameters are summarized in Table 1. The serum insulin level after intranasal insulin delivery with PS-$CaCO_3$ showed a higher C_{max} (403.5 μU/mL) and shorter T_{max} (0.167 h) when compared with intranasal delivery with regular $CaCO_3$. $BA_{0-4\ h}$ of insulin containing regular $CaCO_3$ and PS-$CaCO_3$ was found to be 5.7% and 7.0%, respectively. The serum glucose level after intranasal delivery with PS-$CaCO_3$ was reduced rapidly to its nadir at 0.5 h compared with nasal administration with regular $CaCO_3$, reflecting the difference in the insulin absorption rates. The initial increase of serum glucose level immediately after dosing was deduced to result from the stress of administration (not observed in human studies).

TABLE 1. PHARMACOKINETIC PARAMETERS OF INSULIN (MONKEY)

Route, formulation (n)	*Dose (U per monkey)*	T_{max} *(h)*	C_{max} *(μU/mL)*	$AUC_{0-4\ h}$ *(μU · h/mL)*	*Relative* $BA_{0-4\ h}$ *(%)*	*Glucose nadir level (%)*
Subcutaneous						
Solution (6)	0.5	0.25 ± 0.09	89.1 ± 33.1	91.7 ± 18.5	—	28.5 ± 13.0
Intranasal						
Regular $CaCO_3$ (6)	16	0.33 ± 0.00	223.0 ± 79.7	165.0 ± 57.6	5.7 ± 2.0	38.7 ± 17.7
PS-$CaCO_3$ (6)	16	0.17 ± 0.00	403.5 ± 106.8[1]	206.8 ± 43.4	7.0 ± 1.5	45.6 ± 14.8

Results are expressed as mean ± SD.
[1] $p < 0.01$ compared with $CaCO_3$.

Clinical study

The intranasal delivery of insulin with regular $CaCO_3$ and PS-$CaCO_3$ was investigated in healthy human volunteers. Figure 3a shows the average serum concentration profiles of insulin and serum glucose levels after subcutaneous injection and intranasal administration (16 IU per subject) with regular $CaCO_3$ and with PS-$CaCO_3$. The corresponding pharmacokinetic parameters are summarized in Table 2. Insulin after intranasal administration using both calcium carriers, showed rapid absorption compared with subcutaneous injection. Like with monkeys, in human subjects the absorption behavior of insulin in intranasal delivery using PS-$CaCO_3$ showed a higher C_{max} (17.2 μU/mL), although the AUC was equivalent (24.2 μU • h/mL) when compared with that of regular $CaCO_3$. Figure 3b shows serum glucose levels after intravenous delivery with each calcium carrier at a dose level of 16 IU per subject. In particular, the serum glucose level after intranasal delivery with PS-$CaCO_3$ was reduced rapidly compared with intranasal delivery with regular $CaCO_3$ and subcutaneous administration, reflecting the difference in absorption rates.

Insulin was administered intranasally with PS-$CaCO_3$, which produced a faster absorption rate, at dose levels of 16, 48, and 64 U per subject (Fig. 4a). Insulin absorption after intranasal delivery with PS-$CaCO_3$ increased dose-dependently, maintaining the high absorption rate. $BA_{0-1\ h}$ at each dose remained at the same level (approximately 10%), although $BA_{0-6\ h}$ decreased as the dose increased (Table 2). Figure 4b shows serum glucose levels after intranasal delivery with PS-$CaCO_3$ at insulin dose levels of 16, 48, and 64 U per subject. Serum glucose levels reduced dose-dependently and rapidly. Each serum glucose level reached its nadir within 0.75 h of dosing, and its duration was brief. When insulin was administered intranasally with PS-$CaCO_3$ at a dose level of 64 U per subject, the serum glucose level reached a nadir that was almost equal to that observed after subcutaneous injection (4 U per subject).

DISCUSSION

The nasal route for systemic insulin delivery has received wide attention as an available alternative to the invasive parenteral route of peptide and protein drugs.[2,15,16] The intranasal delivery of peptides and protein drugs as typified by insulin simplifies self-medication, lead-

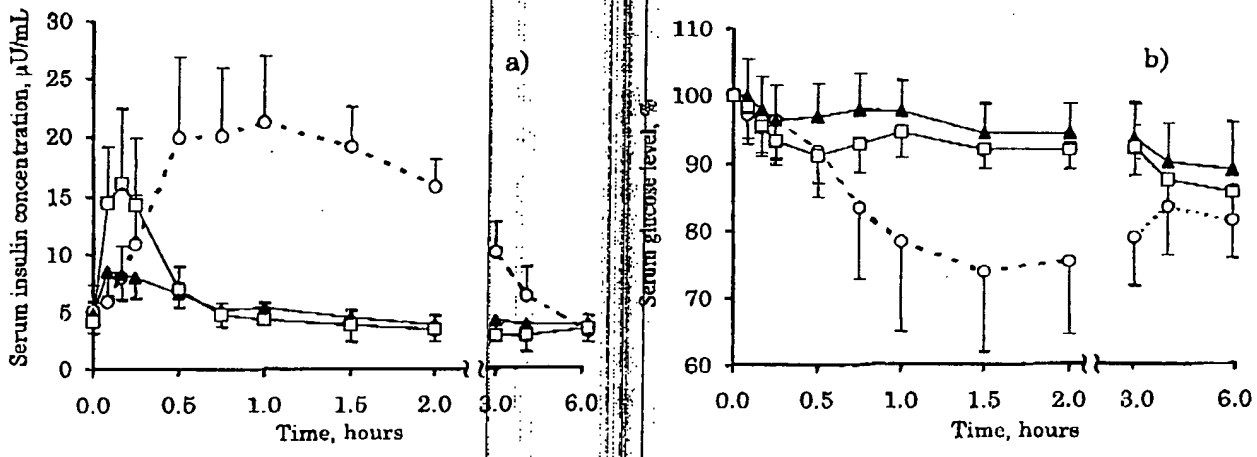

FIG. 3. Serum insulin concentration curves (a) and serum glucose level curves (b) after subcutaneous and intranasal administration with each calcium carrier in humans. Doses of insulin administered subcutaneously and intranasally were 4 and 16 U per subject, respectively. Results are expressed as mean ± SD of more than nine experiments. Serum glucose levels are expressed as the ratio of serum glucose concentration to the initial concentration. Each insulin formulation is expressed as follows: (open circle) subcutaneous formulation; (solid triangle) intranasal formulation with regular $CaCO_3$; (open square) intranasal formulation with PS-$CaCO_3$.

TABLE 2. PHARMACOKINETIC PARAMETERS OF INSULIN (HUMAN)

Route, formulation (n)	Dose (U per subject)	T_{max} (h)	C_{max} (μU/mL)	(μU • h/mL) $AUC_{0-1\ h}$	$AUC_{0-6\ h}$	Relative (%) $BA_{0-1\ h}$	$BA_{0-6\ h}$	Glucose nadir level (%)
Subcutaneous								
Solution (23)	16	1	62.7 ± 20.0	15.8 ± 4.1	64.0 ± 8.8			72.0 ± 11.6
Intranasal								
Regular $CaCO_3$ (12)	16	0.16 ± 0.07	9.2 ± 2.2	6.5 ± 1.1	25.9 ± 8.5	10.3 ± 1.7	10.1 ± 3.3	88.4 ± 4.7
PS-$CaCO_3$ (9)	16	0.13 ± 0.06	17.2 ± 5.7[1]	8.6 ± 2.4	24.2 ± 5.9	13.6 ± 3.8	9.5 ± 2.3	84.9 ± 3.5
PS-$CaCO_3$ (6)	48	0.26 ± 0.12	37.1 ± 20.2	20.9 ± 13.4	38.2 ± 16.3	11.0 ± 7.0	5.0 ± 2.1	78.4 ± 11.9
PS-$CaCO_3$ (6)	64	0.19 ± 0.04	47.5 ± 18.8	23.9 ± 5.9	46.1 ± 3.9	9.5 ± 2.3	4.5 ± 0.4	72.8 ± 10.7

Results are expressed as mean ± SD.
[1] $p < 0.01$ compared with $CaCO_3$ (at 16 U per subject dose level).

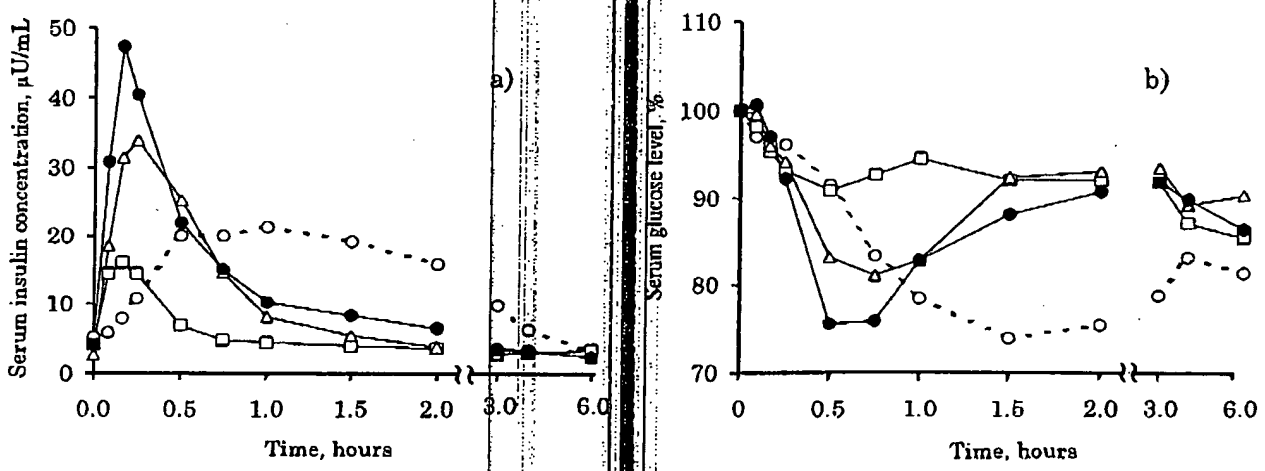

FIG. 4. Serum insulin concentration curves (a) and serum glucose level curves (b) after intranasal administration with PS-$CaCO_3$ at various insulin dose levels in humans. Doses of insulin administered intranasally were 16, 48, and 64 U per subject. Results are expressed as mean ± SD of more than six experiments. Serum glucose levels are expressed as the ratio of serum glucose concentration to the initial concentration. Each insulin formulation is expressed as follows: (open circle) subcutaneously (4 U per subject); (open square) PS-$CaCO_3$ (16 U per subject); (open triangle) PS-$CaCO_3$ (48 U per subject); (solid circle) PS-$CaCO_3$ (64 U per subject).

ing to improved compliance with patients' medical regimens. Most attempts at systemic intranasal delivery have used absorption enhancers because of poor permeability via the nasal mucosa.[11,12] The most important issue in implementing a systemic intranasal drug delivery system for clinical use is the use of a safe and promising drug carrier and/or additives to enable adequate absorbability. Yanagawa[13] suggested that regular $CaCO_3$ is a safe and available drug carrier for the intranasal delivery of various drugs including insulin. Recently, Ishikawa et al.[14] reported that water-insoluble regular $CaCO_3$ powder improved the nasal bioavailability of elcatonin when compared with liquid formulations. They explained that the mechanism of intranasal bioavailability improved by insoluble regular $CaCO_3$ powder was principally achieved by the prolongation of the residence time in the nasal cavity.

We found that PS-$CaCO_3$ is a more promising intranasal carrier for insulin, enabling effective absorption behavior for the treatment of postprandial hyperglycemia in diabetes when compared with regular $CaCO_3$ in both animals and healthy humans. To improve the intranasal insulin absorption, there are several strategies, including the wider distribution of the formulation in the nasal cavity, the greater affinity between the nasal mucosa layer and the formulation, the prolongation of nasal residence time, and the protection of insulin from enzymatic degradation. Effects of PS-$CaCO_3$ on a more effective absorption behavior of insulin were concluded to be the result of a greater affinity between the nasal mucosa layer and PS-$CaCO_3$, which is closely related to its structural characteristics, in addition to the wider distribution of the formulation in the nasal cavity achieved by the administration device.

In clinical studies, $BA_{0-1\,h}$ at each dose maintained the same level, although $BA_{0-6\,h}$ decreased as the dose increased when insulin was administered intranasally with PS-$CaCO_3$ at various dose levels. This result strongly suggested that nasal absorption of insulin was dependent on residence time in the nasal cavity. As shown in the constant $BA_{0-1\,h}$, insulin administered intranasally was absorbed dose-proportionally, while the formulation remained in the nasal cavity. However, once the formulation was removed from the nasal cavity, insulin absorption declined as shown by $BA_{0-6\,h}$. It was suggested that intranasal insulin delivery with PS-$CaCO_3$ is a viable system,

promising constant absorbability and absorption behavior of insulin while the formulation is present in the nasal cavity.

The serum glucose level after intranasal administration with PS-$CaCO_3$ was reduced rapidly in comparison with subcutaneous injection, reflecting the difference on insulin absorption rates. It is suggested that this fast action in the serum glucose level enables insulin medication immediately before or after meals. Furthermore, the short action of the pharmacological effect produced by intranasal insulin delivery suggests a reduced risk of a hypoglycemic side effect due to an excessive duration of serum insulin concentration.

In conclusion, PS-$CaCO_3$ for intranasal insulin delivery is thought to be a promising and safe carrier that can produce a more effective insulin absorption behavior similar to endogenous postprandial insulin secretion in healthy humans. To obtain strong and convincing evidence of the safety of our system, however, we have to implement an additional longer-term toxicity study. We believe that our intranasal insulin delivery system enabling a rapid and short-acting pharmacological effect against postprandial hyperglycemia will be more beneficial than pulmonary insulin delivery systems in the treatment of diabetes.

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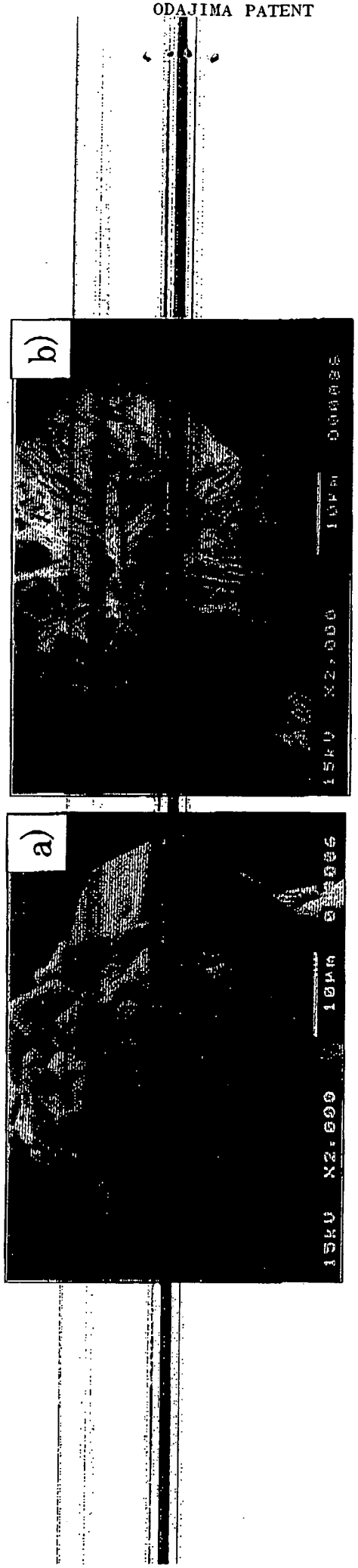
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